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(54) Title: METHOD OF TREATING NEUROLOGICAL DISORDERS

(57) Abstract

This is a method for treating certain neurological disorders in subjects by administering a composition containing a complex of insulin-like growth factor (IGF) and insulin-like growth factor binding protein-3 (IGFBP-3).

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METHOD OF TREATING NEUROLOGICAL DISORDERS

Field of the Invention

This process relates generally to the field of medical therapy and particularly to the treatment of neurological disorders by administering a therapeutic composition containing a complex of an insulin-like growth factor (IGF) and an insulin-like growth factor binding protein (IGFBP).

Background Art

Growth factors are polypeptides which stimulate a wide variety of biological responses (eg. DNA synthesis, cell division, expression of specific genes, etc.) in a defined population of target cells. A variety of growth factors have been identified including transforming growth factor-β_1 (TGF-β_1), TGF-β_2, TGF-β_3, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), insulin-like growth factor-I (IGF-I), and IGF-II.

IGF-I and IGF-II are related in amino acid sequence and structure, with each polypeptide having a molecular weight of approximately 7500 daltons. IGF-I mediates the major effects of growth hormone, and thus is the primary mediator of growth after birth. IGF-I has also been implicated in the actions of various other growth factors, since treatment of cells with such growth factors leads to increased production of IGF-I. In contrast, IGF-II is believed to have a major role in fetal growth. Both IGF-I and IGF-II have insulin-like

activities (hence the name), and are mitogenic (stimulating cell division) for the cells in neural tissue, muscle, reproductive tissue, skeletal tissue and a wide variety of other tissues.

Unlike most growth factors, the IGFs are present in substantial quantity in the circulation, but only a very small fraction of this IGF is free in the circulation or in other body fluids. Most circulating IGF is bound to an IGF-binding protein called IGFBP-3. IGF-I may be measured in blood serum to diagnose abnormal growth-related conditions, e.g., pituitary gigantism, acromegaly, dwarfism, various growth hormone deficiencies, etc. Although IGF-I is produced in many tissues, most circulating IGF-I is believed to be synthesized in the liver.

Almost all IGF circulates in a non-covalently associated ternary complex composed of IGF-I or -II, an IGF specific binding protein termed IGFBP-3, and a larger protein termed the acid labile subunit (ALS). This ternary complex is composed of equimolar amounts of each of the three components. The ALS has no direct IGF binding activity and appears to bind only a preformed IGF-I/IGFBP-3 complex. The ternary complex of IGF + IGFBP-3 + ALS has a molecular weight of approximately 150,000 daltons. This ternary complex is alleged to function in the circulation "as a reservoir and a buffer for IGF-I and IGF-II preventing rapid changes of free IGF." See, Blum, W.F., et al., "Plasma IGFBP-3 Levels as Clinical Indicators", In Modern Concepts in Insulin-Like Growth Factors, (E. M. Spencer, ed., Elsevier, New York) 381-393, (1991).

Nearly all of the IGF-I, IGF-II and IGFBP-3 in the circulation are in complexes, so very little free IGF or IGFBP-3 is detectable. Moreover, a high level of free IGF in plasma is undesirable. It would lead to serious

hypoglycemia because IGF has insulin-like effects on circulating glucose levels. In contrast to the IGFs and IGFBP-3, there is a substantial pool of free ALS in plasma which assures that IGF/IGFBP-3 complex entering the circulation immediately forms ternary complex.

IGFBP-3 is the most abundant IGF binding protein in the circulation, but at least five other distinct IGF binding proteins (IGFBPs) have been identified in various tissues and body fluids. Although these proteins bind IGFs, they each originate from separate genes and have distinct amino acid sequences. Thus, the binding proteins are not merely analogs of a common precursor. Unlike IGFBP-3, the other IGFBPs in the circulation are not saturated with IGFs. None of the IGF binding proteins other than IGFBP-3 can form the 150 Kd circulating ternary complex.

IGF-I and IGFBP-3 may be purified from natural sources or produced by recombinant means. For instance, IGF-I has been purified from human serum for a number of years. See, Rinderknecht, E.W., et al., Proc Natl Acad Sci (USA) 73, 2365-2369 (1976). Recombinant IGF-I processes are shown in EPA 0,128,733, published in December of 1984. IGFBP-3 may be purified from natural sources using a process such as that shown in Baxter et al., "Growth Hormone-Dependent Insulin-Like Growth Factors (IGF) Binding Protein from Human Plasma Differs from Other Human IGF Binding Proteins", Biochem Biophys Res Comm 139, 1256-1261 (1986). IGFBP-3 may be synthesized by recombinant organisms as discussed in Sommer, A. S., et. al., In Modern Concepts of Insulin-Like Growth Factors (E. M. Spencer, ed., Elsevier, New York) 715-728 (1991). This recombinant IGFBP-3 binds IGF-I in a 1:1 molar ratio. The topical administration of the IGF-I/IGFBP-3 complex to rat and pig wounds was significantly more effective than IGF-I alone. Sommer

et. al., ibid. Subcutaneous administration of the complex to hypophysectomized rats "substantially prevents the hypoglycemic effects of IGF-I" administered alone. Sommer et. al., ibid.

U.S. Patent No. 5,093,317 issued on March 3, 1992 to Lewis et. al. discloses a method for using IGF-I to enhance the survival of non-mitotic, cholinergic neuronal cells. The claims do not include the use of IGF binding proteins in conjunction with IGF-I.

Patent Cooperation Treaty Publication No. WO 93/02695, published on February 18, 1993, and applied for by Genentech, Inc. and Auckland Univservices Ltd., discloses a method for treating injuries to or diseases of the central nervous system affecting glial or non-cholinergic neuronal cells with intracerebral infusions of IGF-I or IGF-I analogues.

Patent Cooperation Treaty Publication No. WO 92/19256, published on November 12, 1992 and applied for by Kabi Pharmacia AB, discloses a method for inducing nerve regeneration by treating subjects suffering from neuropathy or degenerative neural disorders with IGF-II or IGF-II + IGF-I. The use of any IGF binding protein in these treatments is not disclosed.

European Patent Application EP 0 308 386 A1, published on March 22, 1989 and applied for by KabiVitrum AB, discloses a method for improving the regeneration of transected peripheral nerves by treating subjects with an effective amount of IGF-I. The use of any IGF binding protein in these treatments is not disclosed.

All of the important elements of the IGF system are found in the central and peripheral nervous systems of humans and other mammals, including IGF-I and -II and the IGF binding proteins and receptors. IGF-I and IGF-II have been implicated in the growth, survival and

differentiated function of several classes of neurons and glial cells.

The presence of IGF-I and IGF-II mRNA and protein in rat and human brain has been well documented. IGF-I and IGF-II mRNAs have characteristic regional distributions in fetal and adult rat and human brain. IGF-I mRNA is present at high levels in the olfactory bulb and cervical thoracic spinal cord and at moderate levels in the midbrain and cerebellum of adult rats. IGF-I mRNA is also synthesized by primary cultures of embryonic astroglial and neuronal cells. IGF-II mRNA is both more abundant in brain than IGF-I mRNA and is much more uniformly distributed. This mRNA is somewhat elevated in the choroid plexus, cerebellum and medulla-pons, and somewhat reduced in midbrain and corpus striatum. In contrast to IGF-I, IGF-II mRNA is synthesized by cultured embryonic astroglial but not neuronal cells. Both mRNAs are highest at embryonic day 8-14 in rat brain, and decline from this peak to the adult level by the time of birth.

IGF-I and IGF-II have also been detected in cerebrospinal fluid (CSF) and by immunohistochemistry in human, rat and cat brain. IGF-II immunoreactivity in the brain is higher than that of IGF-I. Adult and fetal human brain contain both the normal form of IGF-I and a truncated form of IGF-I missing three N-terminal amino acids. IGF-I peptide is also secreted by cultured rat glioma cells. IGF-II immunoreactivity is highest in the anterior pituitary, dorsomedial hypothalamus and supraoptic nucleus of the brain. Larger than normal forms of the IGF-II peptide have been extracted from human brain, and a large form of IGF-II is secreted by cultured explants of neonatal rat brain. The presence of IGF-I and IGF-II mRNA in the brain, and the secretion of these peptides by cultured cells from the central nervous

system suggests that at least some of the IGF found in the central nervous system is produced locally.

The expression of the IGF-I genes in neural tissue is under complex hormonal control. *In vitro* studies indicate that basic fibroblast growth factor (bFGF), which promotes survival of cultured neural tissue, stimulates secretion of IGF-I from cultured fetal rat neuronal and glial cells. In contrast, dexamethasone and retinoic acid, which inhibit the growth of rat glioma cells, reduce the accumulation of IGF-I mRNA and inhibit the secretion of IGF-I peptide by these cells.

Type I IGF receptors, which transduce mitogenic and differentiation signals provided by the IGFs, are also present in the brain. However, the concentration and distribution of this receptor varies during development. In the neonatal rat, brain Type I receptor levels are quite high (4-10 times higher than in the adult), and the receptor is especially abundant in the superficial cortical layers, nucleus accumbens and hippocampus. In the adult rat, Type I receptor levels are reduced and the receptor is more evenly distributed. There is some receptor enrichment in adult superficial and deep cortical layers, olfactory bulb, endopiriform nucleus, basomedial nucleus of the amygdala, thalamic nuclei and hippocampus. Brain Type I IGF receptor is present in two forms, a normal sized form and a somewhat smaller form than that found in peripheral tissues. Apparently this size difference is largely due to reduced glycosylation of the smaller of the brain species. As with IGF-I, bFGF increases the synthesis of Type I IGF-receptors in cultured neuronal and glial cells.

Substantial quantities of the Type II IGF receptor are also found in the brain, but the function of this receptor is obscure. In fetal and early postnatal rats, the Type II IGF receptor is abundant throughout the

brain, whereas it is restricted to neurons in the forebrain (eg. hippocampus and dentate gyrus) in adult rats.

The third element of the IGF system, the IGF binding proteins, are also synthesized by cells of the nervous system and are found in CSF and neural tissue. IGFBP-2 mRNA is abundant in fetal rat brain stem, cerebral cortex, hypothalamus and choroid plexus and persists in adult brain. It is important to note that IGF-II mRNA is also abundant in the choroid plexus, and that this region of the brain is important in generating CSF.

In neonatal and adult rat CSF, IGFBP-2 is the most abundant IGF binding protein, with substantially higher IGFBP-2 levels in neonatal CSF than in adult CSF. Lower levels of IGFBP-3 are also present, as well as traces of lower molecular weight IGFBP species. Using immunocytochemistry with human fetal tissues, IGFBP-3 was localized to neuronal cell bodies in the upper region of the cerebral cortex, while IGFBP-1 and -2 were not detected in the cerebral cortex. IGFBP-3 was not detectable in the meninges.

Cultured fetal, postnatal and adult rat glial and neuronal cells, gliomas and choroid plexus cells synthesize and secrete IGFBP-2, IGFBP-3 and a 24 KDa IGFBP. The glial cultures secreted approximately 5 times as much IGFBP-2 as did the embryonic neuronal cultures, and IGFBP-2 was the only IGFBP secreted by choroid plexus cultures. In contrast, gliomas and astrocytes synthesized predominantly IGFBP-3. The regulation of IGFBP synthesis has not been extensively studied, but it is known that bFGF treatment greatly increases IGFBP secretion by neuronal cultures and inhibits IGFBP secretion in glial cultures. IGF-I

stimulated IGFBP secretion in both cultures and in the rat neuroblastoma cell line B104.

As with many other tissues, IGF treatment of the various cell types of the nervous system leads to both mitogenesis and the expression of tissue-specific differentiated functions. These effects presumably act through the Type I IGF receptors. IGF-I and IGF-II are mitogenic (ie. stimulate cell division) for oligodendrocyte precursor cells from cultured perinatal rat cerebrum explants, embryonic rat sympathetic neuroblasts, human neuroblastoma cells, newborn rat astroglial cells, and neonatal rat cerebral cortex astrocytes.

Furthermore, IGF-I has been shown to promote the survival of various types of cultured nervous system cells. For example, IGF-I acts as a survival factor in cultured embryonic mouse neuroepithelial cells. bFGF, which is mitogenic for these cells, induces endogenous production of IGF-I, which is required for the expression of the mitogenic effect of bFGF. Similarly, autocrine production of IGF-I has been implicated as the mediator of at least part of the mitogenic effect of epidermal growth factor (EGF) on cultured newborn rat astroglial cells. IGF-I has also been shown to protect rat hippocampal and septal neuronal cell cultures from hypoglycemia-induced damage by stabilizing neuronal calcium homeostasis.

In cultures of undifferentiated neural cells, IGF-I promotes the differentiation of oligodendrocyte precursor cells in explant cultures of perinatal rat cerebrum, catecholaminergic precursor cells in cultured chick dorsal root ganglia, and cultured SH-SY5Y human neuroblastoma cells in synergy with the phorbol esters. IGF-I also induces the synthesis of the rat brain glucose transporter gene in primary rat neuronal and glial cell

cultures. Finally, IGF-I, but not IGF-II, significantly increases the potassium-evoked release of acetylcholine (a major neurotransmitter) from adult rat hippocampal tissue slices.

The importance of the IGFs in promoting the growth and function of the nervous system has been demonstrated in a number of *in vivo* studies. Several studies have shown that treatment with IGFs can stimulate neurite outgrowth and synapse formation. For example, both IGF-I and IGF-II substantially stimulate rapid neurite outgrowth in embryonic chick spinal cord motor neurons in culture. Similarly, IGF-II administered daily to mouse gluteus muscle caused rapid, marked terminal and nodal neuronal sprouting of neurites. This effect was detectable after as little as 3 days of treatment and produced 10 fold more neurite sprouts in IGF-II treated than control muscle after one week of treatment. IGF-II treatment also caused a nearly 5-fold increase in the number of endplates that had formed neurite sprouts. This data was interpreted to support the possibility that the IGFs could act as the diffusible factors that are thought to be released by damaged, partially denervated muscle and which lead to increased neurite sprouting by the viable cells that innervate the muscle.

Another set of experiments showed that transgenic mice expressing a human IGF-IA transgene developed substantially larger brains than their control littermates. The brains of the transgenic mice expressing hIGF-I also contained substantially more myelin than did the brains of their control littermates. These effects of IGF-I on brain development occurred with only a modest increase in brain and serum IGF-I levels (1.5-2 fold) and with only a slight and insignificant increase in total body weight. These data demonstrate

the potent effect of IGF-I in stimulating the development of brain tissue and myelination.

Disclosure of the Invention

In accordance with one embodiment of the present invention, there is provided a method for treating a subject for Huntington's disease and Alzheimer's disease, wherein the subject is administered a complex comprising an insulin-like growth factor (IGF) and insulin-like growth factor binding protein-3 (IGFBP-3) in an amount sufficient to alleviate said condition.

In accordance with another embodiment of the present invention, the IGF used in the complex is provided as IGF-I. In a further embodiment, IGF and IGFBP are present in equimolar amounts. In still another embodiment, both IGF and IGFBP-3 are human proteins obtained from recombinant sources.

In accordance with another embodiment of the present invention, the complex of IGF and IGFBP-3 is administered parenterally. In a further embodiment, the complex is administered by subcutaneous injection.

In another embodiment, the subject to whom the complex is administered is a mammal.

In yet another embodiment, the method provides for treating a subject for exposure to neurotoxins, cerebrovascular hemorrhage, neuronal scission during surgery, meningitis or other infection of tissues of the nervous system. The method includes administration of the IGF/IGFBP-3 complex in an amount sufficient to alleviate the condition.

In still other embodiments, the method provides a treatment for multiple sclerosis, amyotrophic lateral sclerosis or Charcot-Marie-Tooth disease, in which the subject is parenterally administered a complex of

IGF/IGFBP-3 in an amount sufficient to alleviate said condition.

The IGF/IGFBP complex can be administered using normal parenteral routes for the treatment of peripheral nervous system disorders or for the treatment of central nervous system disorders in which the blood brain barrier is compromised (eg. multiple sclerosis), thus allowing the passage of complex into the brain. Alternatively, in other conditions, such as Huntington's chorea and Alzheimer's disease, the IGF/IGFBP complex can be administered directly to the CNS by intracranial administration, such as by an implanted shunt into the ventricles of the brain.

While not wishing to be bound by any particular theory, the Inventors propose that the administered complex of IGF and IGFBP-3 results in the gradual release of free IGF in elevated levels. This graded, long lasting increase in bioavailable IGF stimulates the growth, survival and maturation of neuronal tissue cells without causing the local or systemic side effects commonly observed in treatments with free IGF (eg. hypoglycemia, receptor down regulation, growth hormone suppression).

Modes For Carrying Out the Invention

Definitions:

As used herein, "Subjects" are defined as humans and mammalian farm animals, sport animals and pets. Farm animals include, but are not limited to, cows, hogs and sheep. Sport animals include, but are not limited to, dogs and horses. The category pets includes, but is not limited to, cats and dogs.

"Insulin-like growth factor (IGF)" comprises a family of factors, including, but not limited to, IGF-I and IGF-II. IGF is a polypeptide having a molecular

weight of about 7500 daltons. IGF may be obtained from natural sources or prepared by recombinant means.

"Insulin-like growth factor binding proteins (IGFBPs)" comprises a family of binding proteins, including but not limited to IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5 and IGFBP-6. IGFBP may be obtained from natural sources or prepared by recombinant means. At least one form of IGFBP (for example, IGFBP-3) complexes with IGF and with a third molecule known as ALS.

A "therapeutic composition" as used herein is defined as comprising IGF complexed with its binding protein IGFBP-3. The therapeutic composition also contains other substances such as water, minerals and carriers such as proteins.

"Alleviation of the condition" is said to occur when the subject to whom the IGF/IGFBP-3 complex is administered exhibits improved function of affected nervous tissue. For peripheral nervous system conditions, improvements include, but are not limited to, improved coordination of movement, improved muscle function and strength, decreased pain, reduced numbness of extremities, and increased sensory function (eg. touch). For central nervous system conditions, improvements include, but are not limited to, improved ability to reason, improved memory, improved speech, improved coordination or movement, reduced pain and improved sensory function (eg. sight, hearing).

Description of the Invention

The method of the present invention contemplates treating neurological disorders by administering a complex of IGF and IGFBP-3.

Nearly all IGF-I or IGF-II complexes with IGFBP-3. IGF/IGFBP-3 normally circulates in the form of a complex in humans and other mammals. This complex

associates with a third protein (ALS), which is present in excess over the concentration of IGF and IGFBP-3. Therefore, ALS is found both associated with the IGF/IGFBP-3 complex and in the free form. The resultant ternary complex has a size of about 150 kd.

Conditions which are treated by the method of the present invention include Huntington's disease, Alzheimer's disease, exposure to neurotoxins, cerebrovascular hemorrhage, neuronal scission during surgery, meningitis, other infection of the tissues of the nervous system, multiple sclerosis, amyotrophic lateral sclerosis and Charcot-Marie-Tooth disease.

"Huntington's disease" is defined as an autosomal dominant disorder usually beginning in middle age and characterized by choreiform movements and progressive intellectual deterioration. There are estimated to be about 25,000 cases in the United States. It is diagnosed on CT scans by characteristic "boxcar ventricles" which result from atrophy of the caudate nucleus. With that atrophy, there are decreases in levels of the neurotransmitters γ-aminobutyric acid (GABA) and substance P (an 11-amino acid peptide), enkaphalins and dynorphin in the stratum and its sites of projection. However, somatostatin and neuropeptide y may be relatively increased in the caudate nucleus and putamen. Generally there is a pattern of cell death similar to that reproduced experimentally by glutamate receptor agonists that act on the N-methyl-D-aspartate subclass of glutamate receptors. Current treatment is palliative: suppressing choreic movements and agitated behavior with phenothiazine or butyrophenone neuroleptics or reserpine. Administration of IGF/IGFBP-3 helps this condition through its trophic effects on nerve growth and maintenance.

Alzheimer's disease is a form of progressive atrophy of the brain. It is the commonest cause of dementia in the elderly and has a frequency of almost 20% in those over 80 years old. The primary feature is death and disappearance of cells from the brain, resulting in extensive convolutional atrophy. Acetylcholine-transmitting neurons are particularly affected. Loss of peptidergic neurons in the cerebrum is associated with reduced somatostatin and corticotropin releasing factor concentrations. Somatostatin is also abnormally low in the CSF. An early symptom is memory loss, followed by slow disintegration of judgment and affect. The clinician must rule out organic causes, such as drug overdoses, vitamin deficiencies, alcohol, ischemic conditions, etc. At present, management of patients with Alzheimer's disease is largely supportive, limiting the confusion and frustration with their environment. Patients with Alzheimer's disease may improve with IGF/IGFBP-3 complex administration, such as with an improved sense of well-being, affect and/or memory.

Another condition in which the IGF/IGFBP complex promotes healing is exposure to neurotoxins. Polyneuropathy can result from exposure to the following environmental toxins: acrylamide (herbicide, grout), arsenic (herbicide, insecticide), buckthorn, carbon disulfide, diphtheria, dimethylamino propionitrile, γ-diketone hexacarbon solvents, inorganic lead, organophosphates and thallium (rat poison). Many drugs have neurologic adverse reactions. See, for example, Tables 363-1-3, which list polyneuropathies associated with systemic disease, drugs or environmental toxins, and genetically determined conditions, respectively (Harrison's Principles of Internal Medicine, 12th ed. McGraw-Hill, New York City, 1991, pp. 2099-2103). In addition, animal toxins, such as the tetanus toxin and

the toxins of various snakes and scorpions, damage the nervous system and interfere with respiration, heart rate, etc. For each of these toxins, the specific or underlying disorder must be treated, and vital functions may need to be supported. In addition, the administration of the IGF/IGFBP-3 complex speeds healing and encourages the sprouting of new neurites.

Cerebrovascular hemorrhage is characterized by rupture of a cerebral blood vessel and bleeding into the intracranial space, which compresses and may damage cerebral nerve and glial cells. Similarly, during surgery, nerves may be inadvertently or necessarily compressed or severed (scission). In both situations, the administration of IGF/IGFBP-3 will help nervous tissue recover.

Meningitis and other infections of tissues of the nervous system have bacterial and viral origins. Acute viral encephalitis is an acute inflammation of the brain due to virus or hypersensitivity caused by a virus or other foreign protein. If the spinal cord structures also are affected, the condition is called encephalomyelitis. It is frequently called "aseptic" because no organisms are found. Cerebral edema is present, along with numerous small hemorrhages which are scattered throughout the brain, brainstem, cerebellum and sometimes the spinal cord. Viral invasion may cause nerve necrosis and/or inclusion bodies. Demyelinating lesions sometimes are seen around veins. Therapy of the underlying infection is the primary concern. General therapy includes antiviral and/or antibacterial therapy, fluid therapy without overhydration, and if indicated steroid therapy to counteract the swelling associated with meningitis. In addition, IGF/IGFBP-3 administration helps restore nerve and glial cells. IGF/IGFBP can be administered intracranially with other therapies.

Meningitis also is associated with a number of non-bacterial/viral conditions, such as fungal infections (especially with AIDS or immunosuppressive therapy), TB, dissemination of malignant cells as in leukemia, metastatic carcinoma (especially of lung and breast), gliomas, syphilis and sarcoidosis. Current therapy includes treatment of the underlying disorder, as well as steroids (such as prednisone) to reduce inflammation. In addition, administration of IGF/IGFBP-3 enhances recovery of injured nervous and glial tissue.

Multiple sclerosis has been characterized as "a slowly progressive CNS disease characterized by disseminated patches of demyelination in the brain and spinal cord, resulting in multiple and varied neurologic symptoms and signs, usually with remissions and exacerbations." THE MERCK MANUAL, 15th ed., Merck & Co., Rahway, N.J., 1987, pp. 1414-17. There are plaques of demyelination, destroyed oligodendroglia and perivascular inflammation throughout the CNS. Later, nerves also may be destroyed. The administration of IGF/IGFBP-3 will encourage the replacement of neurites and glial cells.

In contrast, amyotrophic lateral sclerosis primarily affects motor neurons, producing muscular weakness and atrophy. Cramps are an early sign. Later fasciculations, spasticity and hyperactive reflexes are observed. IGF/IGFBP-3 administration will encourage the formation of new neurites.

Charcot-Marie-Tooth disease is an autosomally dominant disorder of the peripheral nervous system in which weakness and atrophy, particularly of the peroneal and distal leg muscles, gradually develops over years. Biopsy may show segmental demyelination and remyelination. At present there is no specific treatment, aside from bracing weak muscles such as to

limit foot drop. The administration of IGF/IGFBP-3 is supportive therapy, intended to enhance remyelination.

Systemic administration of IGF and IGFBP-3, either from natural or recombinant sources, as a preformed complex results in the formation of the normal ternary complex with the excess ALS. This type of treatment produces a prolonged increase in the level of circulating IGF, which is gradually released from the ternary complex. This mode of administration avoids the detrimental side effects associated with administration of free IGF-I, namely, hypoglycemia, suppression of growth hormone and ALS production, and release of endogenous IGF-II since administered exogenous free IGF-I replaces endogenous IGF-II in normally circulating IGF-II/IGFBP-3 complexes.

The formulation, method of administration and dosage will depend upon the disorder to be treated and the medical history of the patient. These factors are readily determined in the course of therapy. Suitable patients with neurological disorders can be identified by medical history, physical findings and laboratory tests. The medical history may reveal such facts as loss of coordination, muscle weakness, tremors, dizziness, headache, loss of memory, impaired speech, cognitive difficulties and the specific findings associated with the individual conditions discussed above. Patients may have physical findings such as muscle weakness, impaired reflexes, impaired coordination, disorientation, memory loss, impaired language function, impaired sensory function as well as specific findings associated with the individual conditions discussed above. Indicative diagnostic procedures include computerized tomography (CT) scans, magnetic resonance imagery (MRI), electroencephalography (EEG), cerebrospinal fluid (CSF) analysis and the like.

In accordance with the method of the present invention, the formulation comprises a complex of IGF and IGFBP-3. Preferably, the IGF is IGF-I, although IGF-II also is useful. Because IGF and IGFBP-3 naturally complex in a 1:1 molar ratio, a composition of equimolar amounts of IGF and IGFBP-3 is preferred. The product can be formulated with IGF:IGFBP-3 molar ratios ranging from about 0.5 to 1.5. More preferably, the molar ratio is about 0.9 to 1.3; and most preferably, the product is formulated with approximately a 1:1 molar ratio.

In accordance with the method of the present invention, the IGF and IGFBP-3 are human proteins obtained from natural or recombinant sources. Most preferably, IGF and IGFBP-3 are human IGF-I and IGFBP-3 made by recombinant means and designated rhIGF-I and rhIGFBP-3, respectively. rhIGFBP-3 may be in glycosylated or non-glycosylated form. *E. coli* is a source of the non-glycosylated IGFBP-3. Glycosylated IGFBP-3 may be obtained from Chinese hamster ovary (CHO) cells.

The method of the present invention provides for formulating the complex in modes which are readily apparent to those skilled in the art. Preferably, the IGF and IGFBP-3 are complexed prior to administration to the treated subject. Preferably, the complex is formed by mixing approximately equimolar amounts of IGF-I and IGFBP-3 dissolved in physiologically compatible carriers such as normal saline solution or phosphate buffered saline solution. Most preferably, a concentrated solution of rhIGF-I and a concentrated solution of rhIGFBP-3 are mixed together for a sufficient time to form an equimolar complex.

Depending on the mode of administration, compositions of the complex may be in the form of solid, semi-solid or liquid dosage preparations, such as for

example, tablets, pills, powders, capsules, liquids, suspensions or the like. Physiologically compatible carriers include intravenous solutions, such as normal saline, serum albumin, 5% dextrose, plasma preparations, and other protein-containing solutions. The preferred carrier for parenteral administration of the complex is a sterile, isotonic aqueous solution, such as normal saline or 5% dextrose. Alternatively, a solution of the complex may be placed into an implant, such as an osmotic pump, for the slow release of the complex over an extended period of time. Alternatively, the complex may be provided in sustained release carrier formulations such as semi-permeable polymer carriers in the form of suppositories or microcapsules. See, for instance, U.S. Patent No. 3,773,919 for Microcapsular Sustained Release Matrices Including Polylactides; Sidman et al., Biopolymers 22 (1): 547-556 (1983) for copolymers of L-glutamic acid and γ-ethyl-L-glutamate; Langer et al., J Biomed Res 15: 267-277 (1981) for poly(2-hydroxyethylmethacrylate) or the like.

The mode of administration delivers the complex to the subject in a safe, physiologically effective manner. The complex may be given by intranasal, subcutaneous, intravenous, intramuscular, intraperitoneal, intracranial or other conventional routes of administration. Preferably, the complex is injected subcutaneously, intravenously or intramuscularly. Most preferably, the complex is administered by subcutaneous injection. By subcutaneous injection, the complex appears not to be toxic or mitogenic at the injection site.

The dose of complex to be administered can be readily determined by those skilled in the art, based on the usual patient symptoms discussed above. Preferably, when the complex is administered to humans daily, the

dosage of complex is about 0.01 to 10 mg of IGF-I or IGF-II/kg of body weight/day, complexed to an equimolar amount of IGFBP-3. More preferably, the daily dosage of the complex for humans is about 0.05 to 7.5 mg IGF/kg/day, complexed to an equimolar amount of IGFBP-3. Most preferably, the daily dosage of the complex for humans is about 0.1 to 5 mg IGF/kg/day, complexed to an equimolar amount of IGFBP-3. If daily dosages in excess of about 0.5 mg IGF/kg must be given, the dosage may be divided and injected subcutaneously at two or more sites.

If the IGF/IGFBP-3 complex were administered to humans twice a week, each dose of complex is preferably about 0.05 to 10 mg IGF/kg of body weight, complexed to an equimolar amount of IGFBP-3. More preferably, for twice weekly administration, the dose of the complex is about 0.1 to 7.5 mg IGF/kg, complexed to an equimolar amount of IGFBP-3. Most preferably, for twice weekly administration, the dose of the complex is about 0.5 to 5 mg IGF/kg, complexed to an equimolar amount of IGFBP-3. There is no known upper limit of dosage; however, it is preferable that a single dose not exceed 10 mg IGF/kg of body weight, when the IGF is complexed to an equimolar amount of IGFBP-3. These doses of IGF/IGFBP-3 complex are not expected to cause significant hypoglycemia since the IGF/IGFBP-3 slowly releases IGF to cellular insulin receptors.

Preferably, the patient is started with a relatively low dose of the complex, such as 0.05 mg IGF/kg of body weight/day. Physical examinations, functional tests and diagnostic procedures such as those outlined above should be performed on the treated patients to determine if there is improvement. Preferably, the patient shows improvements in the structure and/or function of the peripheral or central nervous tissue affected by the neurological disorder

following such treatment. If the patient improves with the low dose, the low dose preferably should be continued until acceptable clinical endpoints have been achieved.

If the patient does not respond to low dose IGF/IGFBP-3 complex with sufficient clinical improvement, the dose of complex should be increased gradually until such an outcome is achieved.

The invention has been disclosed by direct description. Following are examples showing the efficacy of the IGF/IGFBP-3 complex in stimulating processes critical to the growth, survival and functioning of neurological tissue. The examples are only examples and should not be taken in any way as limiting to the scope of the process.

EXAMPLES

Example 1

This example is designed to demonstrate the ability of the rhIGF-I/IGFBP-3 complex to stimulate the sprouting of neurites (nerve processes) in cultured embryonic chick spinal cord motor neurons. The sprouting of neurites leads to the re-establishment of innervation and consequently full function of partially denervated neuromuscular junctions and disrupted central nervous system neural connections.

In this experiment, cultures of motor neuron cells are prepared from embryonic chick spinal cord tissue. Dissociated lumbar and brachial spinal cord cells are purified by differential Ficoll gradient centrifugation, and the motor neuron fraction is isolated. Primary cultures of these cells are plated in laminin coated tissue culture dish wells in enriched L15 medium containing 20% horse serum and 20 μg/ml embryonic chick hind limb muscle protein extract. The majority of

the cells in this culture are neurons with large multipolar cell bodies, and non-neuronal cells represent only a few percent of the total. Cells are plated in either the above medium alone, or in medium containing 1 to 100 ng/ml rhIGF-I complexed to an equimolar amount of rhIGFBP-3. The extent of neurite outgrowth in each set of cultures is assessed by light microscopy daily for up to 7 days. The effect of each treatment is determined by measuring the total length of the neurite tree for each neuron at each time point.

Example 2

This example is designed to demonstrate the ability of the IGF-I/IGFBP-3 complex to stimulate regeneration of severed neurons. Traumatic or surgical injury to peripheral nerves is troublesome since the regeneration of damaged nerves is often slow and functionally incomplete. There is a clinical need for agents that can promote more rapid and completely functional regeneration of such damaged nerves.

In this experiment, incisions are made in the hind legs of Sprague-Dawley rats, and the sciatic nerve of each rat is transected at the mid-thigh region. The proximal end of the nerve is placed into one channel of a silicone block containing three channels arranged in a Y-shape. A small, implantable osmotic pump is connected through a piece of tubing to one of the two remaining channels of the block. The third channel is left open, and the block, tubing and pump sutured in place. Finally, the incision is sutured closed.

In one set of animals, groups of rats have the osmotic pump filled with various concentrations rhIGF-I/IGFBP-3 complex (0.1-1 mg/ml of rhIGF-I complexed to an equimolar amount of rhIGFBP-3) in physiological saline plus 1% rat serum albumin (RSA). In a group of control

rats, the pump is filled with physiological saline plus 1% RSA only. The pumps are left in place to pump at a rate of approximately 0.5 μl/hr for approximately 3-4 weeks to allow nerve regeneration.

At the end of the 3-4 week treatment period, the animals are sacrificed and the silicone blocks recovered. The pumps are removed from the block and any tissue in the block is fixed by immersing the opened block in standard glutaraldehyde fixative. The fixed tissue is then stained with osmium tetroxide and dehydrated. Each channel in the block is cut into short lengths numbered starting from the severed nerve stump. Thin sections are cut from each short length of channel and are stained with methylene blue and azur II. Light microscopy is used to evaluate the presence of myelinated axons from the regenerated nerve in each short length of each channel.

Example 3

This example is designed to demonstrate the ability of the rhIGF-I/IGFBP-3 complex to stimulate the growth and myelination of nervous tissue in the central nervous system. A variety of illnesses result in the demyelination of central nervous system neurons (eg. multiple sclerosis, acute disseminated encephalomyelitis). This demyelination results in defective nerve transmission and loss of sensory and motor function. An agent that would stimulate myelin production in such cases would be a useful therapeutic.

In this experiment myelin production is assessed in fetal rat brain aggregate cultures. Whole fetal rat brain cells are dissociated into single cells, filtered, and then placed into aggregate culture medium (Almazan et. al., Dev. Neurosci. 7, 45-54 (1985)) supplemented with 10% fetal calf serum. The cells are

grown in suspension for two days, at which time either fresh medium alone or fresh medium plus 0.01-1 μg/ml rhIGF-I complexed to an equimolar amount of rhIGFBP-3 is added. Myelin produced by the cultures is isolated and quantitated following 10-30 days of culture. At each time point, the number and maturity of oligodendrocytes is also quantitated by measuring the activity of the oligodendrocyte marker 2'-3'-cyclic nucleotide 3'-phosphohydrolase (CNP) in cell homogenates.

This invention has been detailed both by example and by direct description. It should be apparent that one having ordinary skill in this art would be able to surmise equivalents to the invention as described in the claims which follow but which would be within the spirit of the description above. Those equivalents are to be included within the scope of this invention.

CLAIMS

1. A method for treating a subject for Huntington's disease and Alzheimer's disease, said method comprising administering to said subject a complex comprising an insulin-like growth factor (IGF) and insulin-like growth factor binding protein-3 (IGFBP-3), said complex being administered in an amount sufficient to alleviate said condition.

2. The method of claim 1 wherein the complex comprises equimolar amounts of IGF and IGFBP-3.

3. The method of claim 1 wherein the IGF is IGF-I.

4. The method of claim 3 wherein the IGF is recombinant human IGF-I.

5. The method of claim 1 wherein the IGFBP-3 is recombinant human IGFBP-3.

6. The method of claim 1 wherein the IGF is IGF-II.

7. The method of claim 6 wherein the IGF-II is recombinant human IGF-II.

8. The method of claim 6 wherein the IGFBP-3 is recombinant human IGFBP-3.

9. The method of claim 1 wherein the administration is parenteral.

10. The method of claim 1 wherein the parenteral administration is performed by subcutaneous injection.

11. The method of claim 1 wherein the administration is intracranial.

12. The method of claim 1 wherein the sufficient amount is that amount of complex that results in improvements in the structure or function of the nervous system.

13. The method of claim 12 wherein the amount of complex administered is at least about 0.05 mg IGF/kg of body weight/day, said IGF being complexed to an equimolar amount of IGFBP.

14. The method of claim 1 wherein said subject is a mammal.

15. A method for treating a subject for exposure to neurotoxins, cerebrovascular hemorrhage, neuronal scission during surgery, meningitis or other infection of tissues of the nervous system, said method comprising administering to said subject a complex comprising an insulin-like growth factor (IGF) and insulin-like growth factor binding protein-3 (IGFBP-3), said complex being administered in an amount sufficient to alleviate said condition.

16. A method for treating a subject for multiple sclerosis, amyotrophic lateral sclerosis or Charcot-Marie-Tooth disease, said method comprising parenterally administering to said subject a complex comprising an insulin-like growth factor (IGF) and

insulin-like growth factor binding protein-3 (IGFBP-3), said complex being administered in an amount sufficient to alleviate said condition.

17. The method of claim 16 wherein parenterally administering the IGF/IGFBP complex comprises subcutaneous injection.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/13177

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00, 38/16, 38/17, 38/18, 38/30; C07K 14/00, 14/65, 14/475
US CL :514/2, 12; 530/300, 303, 350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 12; 530/300, 303, 350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Journal of Neurochemistry, Vol. 58, Number 4, issued 1992, F.T. Crews et al., "Insulin-like Growth Factor I Receptor Binding in Brains of Alzheimer's and Alcoholic Patients", pages 1205-1210, see entire document.	1-14
Y	WO, A, 93/02695 (GLUCKMAN ET AL.) 18 February 1993, see entire document.	1-17
A	E.M. SPENCER, "MODERN CONCEPTS OF INSULIN-LIKE GROWTH FACTORS", published 1991 by Elsevier (N.Y.), pages 715-728, see entire document, especially page 727 and Figure 14.	1-17

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
02 FEBRUARY 1995

Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/13177

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A, 92/19256 (LAKE ET AL.) 12 November 1992, see entire document.	1-17
Y	US, A, 5,187,151 (CLARK ET AL.) 16 February 1993, see entire document, especially the Abstract and Column 4, lines 45-52.	1-17
Y	US, A, 5,258,287 (BAXTER ET AL.) 02 November 1993, see entire document, especially the Abstract and Column 3, line 67 - Column 4, line 4.	1-17
Y	US, A, 5,093,317 (LEWIS ET AL.) 03 March 1992, see entire document.	1-17
Y	US, A, 5,068,224 (FRYKLUND ET AL.) 26 November 1991, see entire document.	15-17
Y	Journal of Neuroscience, Vol. 12, Number 4, issued April. 1992, R.C. Armstrong et al., "Pre-oligodendrocytes from Adult Human CNS", pages 1538-1547, see especially the Abstract and pages 1542-1545.	16-17
Y	Proceedings of the National Academy of the Sciences USA, Volume 83, issued February 1986, F.A. McMorris et al., "Insulin-like Growth Factor I/Somatomedin C: A Potent Inducer of Oligodendrocyte Development", pages 822-826, see entire document.	16-17
Y	Journal of Cell Biology, Volume 111, issued September 1990, R. Armstrong, "In Vitro Analysis of the Oligodendrocyte Lineage in Mice During Demyelination and Remyelination, pages 1183-1195, see entire document.	16-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/13177

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

STN files: biosis, medline, embase, ca, wpids
search terms: igf##, insulin-like growth factor?; igfbp##, insulin-like growth factor binding protein#; alzheimer##, huntington##, peripheral nervous system, central nervous system, als, amyotrophic lateral sclerosis, multiple sclerosis, Charcot-Marie-Tooth disease, neuropath###, neurotoxi#, ischemia, cerebral hemorrhage, treatment, therapy